with the characteristics of *Metacamopiella* above; therefore, a new synonym of *M. euzeti* Kohn, Santos, and Lebedev, 1996, is proposed. The examination of type material of *M. oligoplites* allowed comparisons with the original description and illustrations by Takemoto et al. (1996) to be made: (1) the papillalike structures in the vaginal ducts were not described, and (2) the description and illustration of the midsclerite of the clamp were incomplete. Observation of the paratype illustrated by Takemoto et al. (1996) showed that the midsclerite bifurcates at their extremities and is not bifurcated only in the anterior extremity as illustrated by these authors.

Metacamopia Lebedev, 1972 is characterized by the presence of sclerotized structures in the vaginal ducts. Because the specimens described by Takemoto et al. (1996) did not show this character, the authors emended the diagnosis of *Metacamopia* to accommodate their specimens, including in this genus the species with or without sclerotized structures in the vaginal ducts. This emended diagnosis is not valid, because it was based on insufficiently studied material. As mentioned above, the specimens described in Takemoto et al. (1996) do not have sclerotized structures in the vaginal duct but have papillalike thickened structures, as in *Metacamopiella*. Also, the position of the vagina (ventral, as in *Metacamopiella*) annotated by Takemoto et al. (1996) does not correspond to the original diagnosis (dorsolateral) of *Metacamopia* made by Lebedev (1972).

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Research Note

Histochemistry and Ultrastructure of the Metacercarial Cyst of *Cryptogonimus chyli* (Trematoda: Cryptogonimidae)

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ABSTRACT: Histochemical and ultrastructural studies were conducted on metacercarial cysts of the cryptogonimid trematode *Cryptogonimus chyli* from the skeletal muscles of the fantail darter *Etheostoma flabellare*. Metacercarial cysts were composed of an outer host capsule and an inner parasite cyst. The host capsule contained an outer region of fibrocytes, collagen, and lymphocytes and a thin inner layer. The parasite cyst was a uniformly thin and homogeneous layer. The host capsule stained strongly for connective tissues and protein and moderately for lipids, nucleic acids, nonspecific esterase activity, and acid and alkaline phosphatase activities. The parasite cyst stained intensely for acid mucopolysaccharides and moderately for neutral

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mucopolysaccharides and proteins. Although the metacercarial cysts of the related cryptogonimid, *Bolbogonotylus corkumi,* are also located in the muscle tissue of fantail darters, differences are apparent in the host and parasite response.

KEY WORDS: *Bolbogonotylus corkumi,* electron microscopy, glycocalyx, mitochondria, fantail darter, fibrocyte.

Adults of *Cryptogonimus chyli* Osborn, 1903 are intestinal parasites of several North American fish species (Hoffman, 1967; Margolis and Arthur, 1979; Font, 1987). Small fish serve as second intermediate hosts, and several species of darters (Font, 1987) harbored the parasite at our collection site in O'Neil Creek near Eagleton, Chippewa County, Wisconsin (91°45′00″N, 44°95′00″W). In this study, metacercariae were obtained from fantail darters, *Etheostoma flabellare* Rafinesque.

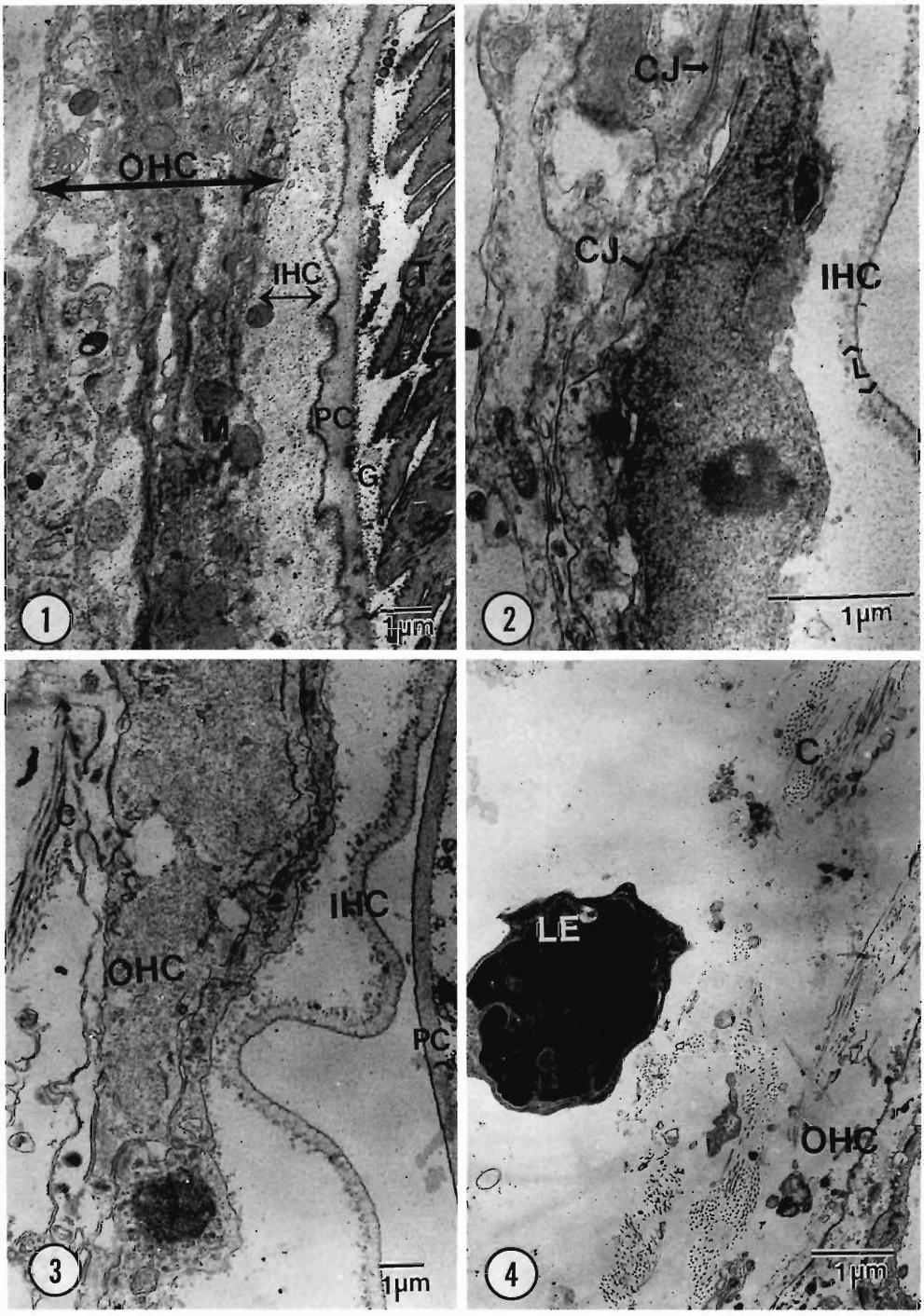
There have been a number of ultrastructural studies on the metacercarial cysts of digenetic trematodes from fishes (Sogandares-Bernal and Lumsden, 1964; Stein and Lumsden, 1971; Mitchell, 1974; Higgins et al., 1977; Halton and Johnston, 1982; So and Wittrock, 1982; Wittrock et al., 1991). Walker and Wittrock (1992) were the first to describe the metacercarial cyst of a member of the Cryptogonimidae in their study of *Bolbogonotylus corkumi* Font, 1987. The host used as the source of metacercariae for *B. corkumi* was also the fantail darter. Here, we describe the composition of the metacercarial cyst of *C. chyli* and compare our findings with what was previously described for the metacercarial cyst of *B. corkumi.*

The collection and processing of parasites and host tissues for histochemistry and transmission electron microscopy were as described by Walker and Wittrock (1992). Many of the fantail darters were infected with hundreds of *C. chyli* metacercariae. Whereas the metacercariae of *B. corkumi* were encysted only in skeletal muscle tissue, *C. chyli* metacercariae were distributed throughout a number of organs, including adipose and connective tissue, kidney, liver, and skeletal muscle. However, the majority of cysts were located within the muscle tissue. For subsequent comparisons of *C. chyli* with *B. corkumi,* the results of this study pertain only to those metacercariae encysted within skeletal muscle. Voucher specimens of *B. corkumi* (HWML 39821) and *C. chyli* (HWML 39820) were deposited in the Harold W. Manter Laboratory of Parasitology at the University of Nebraska State Museum.

Fifteen metacercarial cysts averaged 113 by 123 μm in diameter. The cyst wall varied in thickness from 2.0 to 9.2 μm ($\bar{x}$ = 3.2 μm). The cyst wall consisted of an outer host capsule and an inner cyst of parasite origin (Fig. 1). The host capsule stained intensely with Gömöri trichrome and Pollak rapid trichrome, indicating the presence of collagenous connective tissue. This layer was also strongly reactive with mercuric bromphenol blue and Sudan black B and moderately reactive with oil red O and azure B, suggesting the presence of a lipoprotein component. The parasite cyst stained moderately with the periodic acid–Schiff test (PAS) and intensely with alcian blue (pH 2.5), indicating the presence of both neutral and acid mucopolysaccharides. This layer also stained moderately with bromphenol blue, suggesting the presence of proteinaceous material.

The outer host capsule contained fibrocytes, unidentified cells, and scattered patches of collagen, which demonstrated its typical periodicity (Figs. 2, 3). Large numbers of fibrocytes but only an occasional lymphocyte were observed in the outer host capsule (Fig. 4). Numerous mitochondria, presumably from degenerating cells, were distributed throughout the entire host capsule (Fig. 1). The inner host capsule was often separated from both the outer host capsule and the parasite cyst (Fig. 3). This portion of the host capsule contained a row of oblong, lightly stained spheres that rested upon a thin electron-dense layer (Fig. 2). The parasite cyst was very uniform in size and structure with a thickness of <1 μm (Fig. 3). Similar to the inner host capsule, the outer parasite cyst consisted of a thin electron-dense layer that was more dense than that of the inner host capsule (Fig. 3). The major portion of the parasite cyst was composed of a homogeneous, light-staining material (Figs. 1, 3). Sloughed debris and glycocalyx were present between the parasite cyst and tegument (Figs. 1, 3).

Metacercarial cysts of *C. chyli* and *B. corkumi* were compared from fantail darter skeletal muscle. Although the cyst composition in these 2 species was similar, there were a number of differences. The host capsule was much thinner in *C. chyli,* particularly the zone of compacted, degenerative cells within the inner host capsule.



Figures 1–4. Transmission electron micrographs of the metacercarial cyst wall of *Cryptogonimus chyli*. **1.** Tegument (T) enclosed by thin, lightly stained parasite cyst (PC). Note glycocalyx (G) between the tegument and parasite cyst. Lightly stained material and some cell debris, such as mitochondria (M), form the inner host capsule (IHC). Unidentified and degenerative cells form the outer host capsule (OHC). **2.** An apparent lipid layer (L) occurs at the inner boundary of the inner host capsule. Note the fibrocyte (F) within the outer host capsule. Cell junctions (CJ) occur irregularly throughout the outer host capsule. **3.** Collagen (C) is occasionally scattered throughout the outer zone of the outer host capsule. Note separation of the inner host capsule from both the parasite cyst and outer host capsule. **4.** Lymphocyte (LE) and collagen (C) on external margins of the outer host capsule.

The spheres of the inner host capsule in *B. corkumi* occurred in a multiple rather than a single layer, as observed in *C. chyli* (Walker and Wittrock, 1992). These spheres were tentatively identified as lipid droplets because of their physical appearance and the positive reaction of oil red O and Sudan black B in this region. In *B. corkumi,* the dense layer of cells compacted against the parasite cyst probably prevented the host layers from separating. The lack of a dense inner host capsule in *C. chyli* probably allowed separation of the host layers. This detachment may be occurring, however, only after processing of tissues. Nevertheless, these results suggest a higher degree of structural rigidity or compactness of the *B. corkumi* cyst.

In *B. corkumi,* granulocytes, probably functioning as macrophages, were occasionally observed in the outer host capsule, whereas only an occasional lymphocyte was identified in C. *chyli.* For both species, many cysts were studied from several darters, but relatively few granulocytes were observed at the ultrastructural level. However, metacercarial cysts of both species stained with moderate intensity for acid and alkaline phosphatase activities and for nonspecific esterase activity. Macrophages demonstrate all 3 enzyme activities (Pearsall and Weiser, 1970; Vernon-Roberts, 1972; Ross and Reith, 1985). In teleosts, granulocytes stain positively for both alkaline and acid phosphatases (Hine, 1992). Therefore, the host's immune response probably is directing phagocytic activity against the cysts, but at the ultrastructural level it is difficult to observe macrophages, much less ascertain the intensity of macrophage infiltration. Moreover, the lack of identified granulocytes in our ultrastructural observations of *C. chyli* may result from the host directing phagocytic activity against the much larger cyst of *B. corkumi.* This granulocytic activity in *B. corkumi* could certainly be the result of its larger cyst size and the much greater quantities of fibrocytes and cell debris.

Many more fibrocytes and collagen fibers were observed in the host capsule of *B. corkumi* than in *C. chyli* in all the cysts studied. Additionally, an increased staining reaction for the Gömöri and Pollak stains implies greater deposits of collagenous connective tissue in *B. corkumi.* This finding suggests differential host responses to *B. corkumi* and *C. chyli* even though they are closely related species.

Another apparent difference between the 2 cryptogonimids is the relatively small amount of glycocalyx covering the tegument of *C. chyli* as compared with the thick layer of glycocalyx encompassing *B. corkumi* (Walker and Wittrock, 1992). The glycocalyx, which is an acid mucopolysaccharide and stains with both alcian blue and PAS, is commonly observed surrounding the encysted trematode. Numerous cysts of both species were observed, and relatively little material stained between the tegument and parasite cyst of *C. chyli* as compared with *B. corkumi.* Lumsden (1975) hypothesized that the glycocalyx, in one of its properties, plays a protective role against the host immune response. The question is whether the reduced host activity is in response to the lower levels of glycocalyx secreted by *C. chyli* or whether because of a weak host response, less glycocalyx is needed to protect the parasite from its host. Moreover, could the apparently weaker host response be simply related to the much smaller size of the *C. chyli* cysts, because less damage would occur with these metacercariae? These are difficult questions to answer without more detailed studies of host responses to encysted parasites.

This study demonstrates that, as has been noted by others, one should not generalize too much on the structure and chemical composition of metacercarial cysts (Huffman, 1987; Wittrock et al., 1991). Even between closely related species infecting the same host in identical tissue, there are differences in both the host and the parasite responses.

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Research Note

Distribution and Prevalence of *Alloglossoides caridicola* (Trematoda: Macroderoididae), a Parasite of the Crayfish *Procambarus acutus* Within the State of Louisiana, U.S.A., and into Adjoining States

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ABSTRACT: A survey of 765 crayfish conducted during April and May 1998 indicated that *Alloglossoides caridicola* infected the antennal glands of *Procambarus acutus* from a variety of habitats within Louisiana, north of the coastal marsh region. Infections were absent from crayfish collected in the prairie region of southwestern Louisiana but extended into eastern Texas, southern Arkansas, and southwestern Mississippi. Prevalence of infection was 0% at 22 localities but ranged from 5.6% to 100% at 23 localities.

KEY WORDS: Trematoda, Macroderoididae, *Alloglossoides caridicola,* distribution, prevalence, crayfish, *Procambarus acutus,* Louisiana, Texas, Arkansas, Mississippi.

Alloglossoides caridicola was described by Corkum and Turner (1977) from the antennal glands of crayfish, *Procambarus acutus* (Girard, 1852), collected near Rosedale, Louisiana. Although its life cycle is unknown, *A. caridicola* is unusual in that it attains sexual maturity and reproduces in an invertebrate. No other hosts have been reported for the worm, nor has it been reported from other localities.

In April and May 1998, I undertook a survey of the distribution and prevalence of *A. caridicola* within Louisiana and the contiguous border areas of adjoining states (eastern Texas, southern Arkansas, and southwestern Mississippi, U.S.A.). Crayfish were taken by dip net from 45 localities (Table 1) representing a variety of aquatic habitats and were identified according to descriptions in Hobbs (1972, 1981). Paired antennal glands of 668 *P. acutus* and 97 *P. clarkii* (Girard, 1852) were removed, dissected under a stereomicroscope, and examined for presence of worms. Representative voucher specimens of *A. caridicola* from 23 localities were fixed in hot alcohol-formalin-acetic acid, stained in Semi-